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VARIATION IN THE DIPHTHERIA GROUP

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The correct interpretation of the morphologic characters of the various organisms roughly classified under the "Diphtheria Group" has long interested bacteriologists. After the discovery of *B. hofmanni* by Hofmann-Wellenhof in 1887, many other bacilli have been reported which resemble B. diphtheriae so closely morphologically that many believe that these so-called pseudodiphtheria bacilli belong with the true diphtheria bacillus in one family. Some go so far as to assert that there is a transition or variation under certain conditions from the typical virulent diphtheria bacillus to the atypical nonvirulent pseudodiphtheria bacillus. A few believe that a change takes place in the opposite direction. Such theories necessarily imply that the organism which causes clinical diphtheria is subject to wide variation in its morphology as well as in its virulence. If there is variation in these characters it is natural to infer that its other biochemical characters may vary also. The present study was undertaken in an attempt to answer the following questions:

1. Do members of the diphtheria group change their morphology?
2. Do members of the diphtheria group vary in their ability to ferment carbohydrates?
3. Do members of the diphtheria group vary in their virulence?
4. Is variation in one of these characters correlated with variation in any of the others?

I. MORPHOLOGY

The morphologic resemblance of the avirulent pseudodiphtheria bacillus to the virulent diphtheria bacillus shows that if these forms belong to two distinct species, their differentiation by the microscopic examination of stained preparations is impossible. Indeed, cultures of virulent diphtheria bacilli often present distinctly different morphologic pictures. Cultures from different parts of the United States are reported to vary in their morphology.[1] Cultures obtained by me

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[1] Jour. Mass. Boards of Health, 1902,-3, 12, p 74.

from cases of diphtheria in the epidemic at Newport, R. I., during July and August, 1917, showed large bacilli of the A A^1 B^1 C^1 type of Wesbrook's classification almost exclusively, while Providence (R. I.) cultures at the same period showed almost exclusively C^1 D^1 D^2 varieties. Hence the morphology of true diphtheria bacilli obtained from cases in localities very near each other may differ to a considerable degree. The granular types of the diphtheria bacillus are relatively infrequent in Providence. If the presence of granules in the diphtheria bacillus was considered necessary for a positive diagnosis, as Rickards[2] (1908) maintains to be the case in Boston, cultures of persons in Providence harboring virulent barred diphtheria bacilli would be reported as negative with considerable danger to the public health.

Variation in the morphology of an individual culture of B. diphtheriae is an entirely different matter from variation among cultures from different sources. If cultures of B. diphtheriae vary individually in their morphology it is of the greatest importance that this be considered in the routine laboratory diagnosis. The work of Denny[3] shows that the diphtheria bacillus has a definite evolutionary cycle during a 24-hour incubation at 37 C. Wherry[4] shows that a typical diphtheria organism may never, under certain conditions, reach a granular stage of development. The various conditions which figure in the determination of the types assumed by cultures of the diphtheria bacillus are little understood, but the chemical composition of the medium and especially its reaction may be of importance. Since dextrose, a sugar fermented by the diphtheria bacillus, is added to Loeffler's blood serum medium a constant change in reaction may be taking place as the bacteria grow. It is very improbable that exactly the same amounts of acid are produced in different parts of the same tube or in a series of tubes to which a culture may be transferred, and these variations in reaction may have some effect on type formation. It is evident that the conditions under which a pure culture of B. diphtheriae is growing may differ in a great variety of ways and it is a question whether these various diverse conditions do not cause a variation in type. This question is the subject of the following study.

The cultures used were obtained from 3 sources, Newport, R. I., Providence, R. I., and New York City. Cultures numbered 1 to 11 were obtained from the throats of persons infected with diphtheria during an epidemic at

[2] Am. Jour. Pub. Hyg., 1908, 18, p. 272.

[3] Jour. Med. Research, 1903, 9, p. 117.

[4] Influence of Oxygen Tension on Morphologic Variations in B. Diphtheriae, Jour. Infect. Dis., 1917, 21, p. 47.

Newport in July and August, 1917. McCoy, Bolten and Bernstein[5] report that while this epidemic was of an "explosive" and widespread character there were very few severe cases of diphtheria.

Cultures 12-14 were obtained at the Providence City Hospital from the throats of persons infected during the epidemic at Newport.

Cultures 15-18 were obtained from cultures sent to the Providence Health Department for diagnosis. The cases of diphtheria occurring in Providence during the past year have been of unusual severity.

Cultures 19-25 were isolated from throat cultures obtained from the New York City Department of Health.

Before making any investigation with respect to variation in the morphology of the cultures they were plated on agar and colonies fished and replanted on Loeffler's serum medium. This process was repeated when there was any doubt of the absolute purity of the culture.

The following method was used in testing for variation in morphology: Each of the cultures was transferred to a fresh, moist Loeffler's serum slant and incubated 20 hours at 37 C. Permanent microscopic preparations were then made of each culture stained with Loeffler's methylene blue. The 25 cultures were then plated on dextrose blood serum agar and incubated 48 hours at 37 C. Each culture was so diluted that well isolated colonies would appear on the medium and the dilutions were so prepared that as far as possible each colony represented the progeny of a single organism. Five single colonies were fished from the plates of each culture and transferred to Loeffler's serum slants by inoculating the water of condensation and flowing it over the surface of the medium. The 125 tubes thus obtained were incubated 20 hours at 37 C. and a smear was then prepared from each tube. In making this smear care was taken to get a composite sample of the whole growth on the surface of the slant. All the types of B. diphtheriae occurring in each smear were recorded for future reference. One tube from the 5 prepared from each culture was selected for replating. Selection was made either because there was an approach toward a pure type culture or because types appeared which were not present in the original culture. This process of plating a selected culture and fishing 5 isolated colonies was continued through 10 successive platings when growths on blood serum from 50 colonies of each of the 25 cultures had been examined. At the end of the examination 250 permanent mounts of the selected cultures had been made while nearly 1,000 smear preparations which had not been permanently mounted had been examined for types present.

Table 1 shows the percentage of frequency of type in the progeny of the 25 original cultures. This table was derived by counting the number of times that each of Wesbrook's types appeared in the tubes obtained from each of the 25 original cultures and dividing this number by the number of opportunities for each type to appear. Enough measurements of types were made and compared with the measurements reported by Wesbrook[6] (1900) to make certain that the proper letters were assigned to the types present in the cultures.

[5] Pub. Health Rep. U. S. Mar. Hosp. Serv., 1917, 32, p. 1787.

[6] Tr. Assn. Am. Phys., 1900, 15, p. 198.

TABLE 1

PERCENTAGE OF FREQUENCY OF TYPE IN THE PROGENY OF 25 CULTURES

Culture Number	Original Type	A	A^1	A^2	B	B^1	B^2	C	C^1	C^2	D	D^1	D^2	E^2
1	ACC^1DD^1	2	2	0	8	6	0	34	51	6	57	77	53	0
2	$A^1B^1CC^1$	10	6	0	8	8	0	50	65	15	50	71	34	0
3	DD^1D^2	6	6	4	2	8	0	40	85	29	19	61	51	0
4	C^2	0	0	2	0	0	0	0	0	100	0	0	8	0
5	DD^1D^2	2	2	0	0	4	0	35	60	12	43	84	67	0
6	$C^1D^1D^2$	4	2	2	0	0	0	42	85	19	38	76	57	0
7	$AC^1C^2D^2$	6	0	2	0	0	0	6	45	87	2	18	14	0
8	$C^1D^1D^2$	0	0	0	0	0	0	26	34	2	38	85	85	0
9	C^2D^2	0	0	0	0	0	0	0	4	0	15	57	95	0
10	$C^1D^1D^2$	2	2	0	2	2	0	57	73	12	59	67	44	0
11	$C^1D^1D^2$	0	2	0	0	0	0	12	44	16	8	93	87	0
12	D^2	0	0	0	0	0	0	0	0	0	0	0	83	16
13	$B^1C^1C^2D^2$	0	0	0	0	0	0	0	6	10	0	18	91	4
14	$A^1B^1C^1D^1$	10	2	0	0	8	0	18	85	37	18	77	66	0
15	CC^1DD^1	0	0	0	0	0	0	6	41	18	12	91	85	0
16	D^1D^2	8	0	0	2	2	0	21	73	21	21	88	65	0
17	$AA^1C^1D^1$	8	0	0	0	0	0	6	55	21	17	82	87	0
18	$C^1C^2D^1D^2$	0	2	0	0	0	0	6	61	72	6	51	68	0
19	D^1D^2	0	0	0	0	0	0	0	0	2	0	44	100	0
20	D^2	0	0	0	0	0	0	0	6	8	0	54	100	0
21	D^1D^2	0	0	0	0	0	0	0	12	4	0	43	100	0
22	D^1D^2	0	0	0	0	0	0	0	8	2	0	40	97	0
23	D^1D^2	0	0	0	0	0	0	0	0	0	0	17	100	0
24	$C^1DD^1D^2$	6	0	0	2	0	0	46	79	14	40	85	59	0
25	D^2	0	0	0	0	0	0	0	0	6	0	6	100	0

Several interesting facts are shown by Table 1. The various forms of the A and B types are relatively infrequent in the progeny of the 25 original cultures even when an A or B type was present in the culture used for the first plating. Types of the C and D groups are the ones which predominate over all others while E^2 was the only representative of the smaller forms of B. diphtheriae which appeared in any of the series of cultures. Cultures 3, 5, 16, 19, 20, 21 and 25 indicate that although a culture may show only forms of the D group of Wesbrook's classification, its progeny may develop forms of the C group. Cultures 3, 5 and 16 produced a large number of the C group forms while the others showed only a few. Another surprising fact brought out by the table is that cultures of the solid staining C^2 and D^2 types may show in later growth barred and even granular types in greater or less abundance. Cultures 9, 20 and 25 produced various forms of the C and D groups which were not present in the original culture and which would not be expected to occur in a pure culture of a solid staining type. Cultures 13, 21, 22 and 23 showed a gradual replacement of the barred types by the solid staining types.

A fair definition of variation in type would be that the predominating types present in subcultures were different from the types present

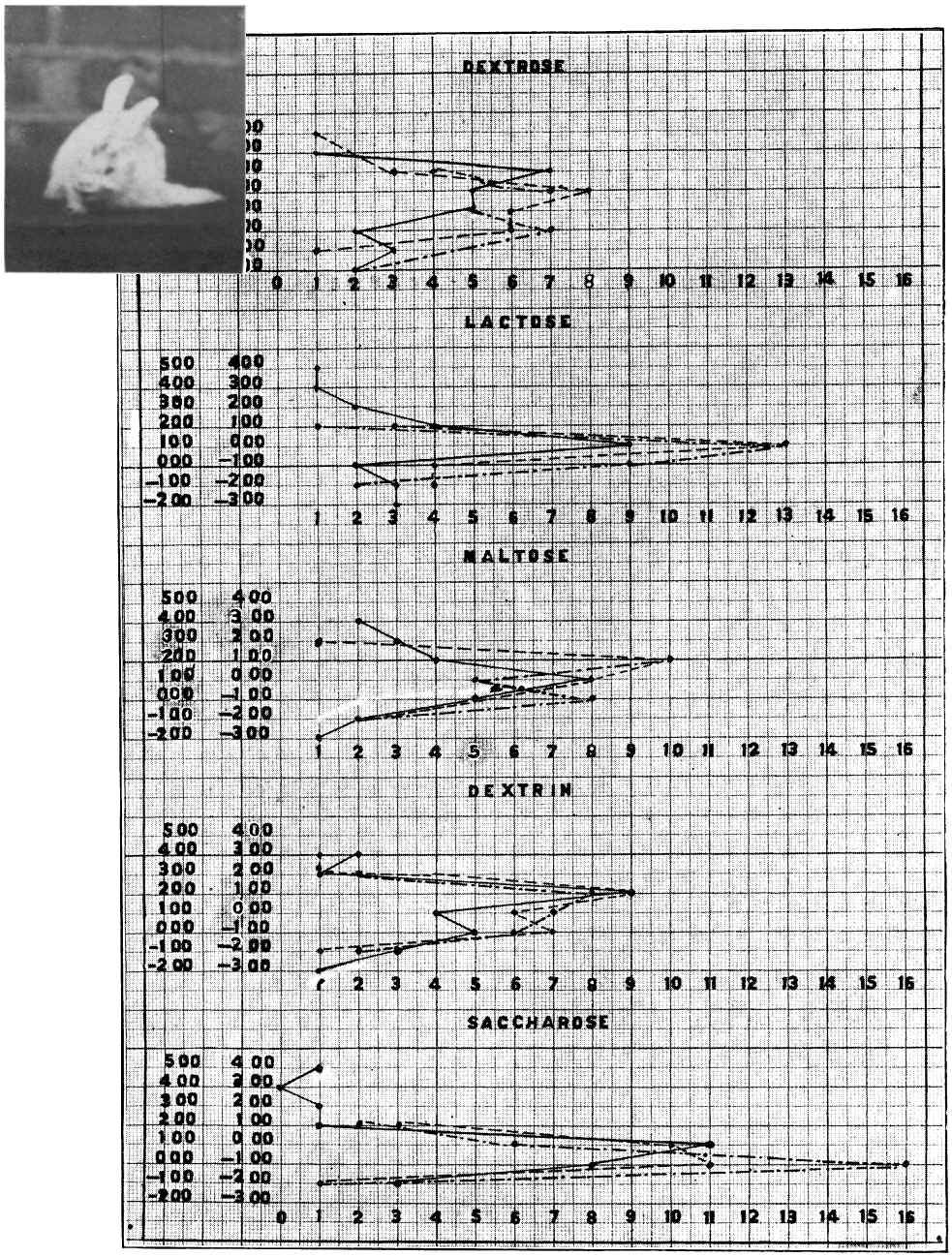

Chart 1.—Frequency curves of fermentative reactions of the 25 original cultures and after the fifth and tenth platings.

in the original culture. Williams[7] using this criterion found that none of the cultures examined had varied. The types seemed rather to revolve around a mean so that transfers from each culture under examination showed types characteristic of the first culture examined.

Using this criterion of variation as a standard a study of Table 1 shows that some of the cultures have varied while others have not. Cultures 1, 4, 6, 7, 8, 11, 12, 18, 19, 20, 21, 22 and 25 show enough of the original types appearing in the later tubes to justify the statement that they have remained reasonably true to type even after 10 platings. Cultures 2, 3, 5, 9, 10, 13, 16 and 17 show by the percentage of types present in the subcultures examined that a distinct variation in type has resulted from the selection of different colonies for replating. There is reasonable doubt whether cultures 14, 15, 23 and 24 show real variation. It appears from this analysis of the results of the experiment described above that 8 members of the diphtheria group have shown morphologic variation, 4 may have varied only slightly, if at all, while 13 showed no reasonable indication of variation. It would seem that some cultures have the ability to vary in morphology while others lack this characteristic.

II. STUDY OF FERMENTATION

The power of bacteria to break down carbohydrates with the production of acid is in many cases a valuable means of classification. We are by no means certain, however, that the testing of the fermentative reactions of members of the diphtheria group has any real value for diagnosis. To be of diagnostic value the test must separate the virulent diphtheria bacilli from those true diphtheria bacilli which have lost their virulence as well as from the so-called "pseudodiphtheria" organisms. A survey of the work done by different investigators would lead one to conclude that fermentative reactions are of practically no value for diagnosis. Fermentative reactions may, however, be of considerable value in classifying the group.

Knapp[8] and Zinsser[9] found that B. diphtheriae fermented dextrose, maltose and dextrin, but not saccharose, while B. xerosis fermented dextrose, maltose and saccharose, but not dextrin. B. hofmanni failed to ferment any of the carbohydrates. Moshage and Kolmer[10] found that fermentative reactions were too irregular to be of use in determining the virulence of a culture of diphtheria bacilli. The following

[7] Jour. Med. Research, 1902, 8, p. 83.
[8] Jour. Med. Research, 1904, 12, p. 475.
[9] Jour. Med. Research, 1907, 17, p. 277.
[10] Jour. Infect. Dis., 1916, 19, p. 19.

study was undertaken to determine whether subcultures varied in their fermentative power from the cultures from which they were obtained.

The 25 cultures described in the previous section of this study were used and fermentation reactions were tested before the first plating, after the fifth and after the tenth platings on each of the cultures. Sugar-free nutrient broth prepared according to the Standard Methods of the American Public Health Association (1912) was used to which was added 1% of the various carbohydrates (Merck's). The medium was sterilized at 15 lbs. pressure for 15 minutes since Mudge[11] has shown that sugar mediums so sterilized are hydrolyzed less than when sterilized in the Arnold. All fermentation tests were made in triplicate and 3 control tubes were incubated and tested with each set of tubes. The period of incubation at 37 C. for maximum fermentation with the carbohydrates used in this study was determined as Morse[12] had previously done. It was found that the maximum acidity was produced in dextrin broth on the 8th day, in saccharose broth on the 9th day, in dextrose and maltose broth on the 12th day and in lactose broth on the 14th day of incubation. Throughout the study of fermentation tests to be described titrations were made on the days indicated by this preliminary test. Five c c amounts of the cultures to be tested were titrated according to the Standard Methods of the American Public Health Association using phenolphthalein as an indicator. All titrations were properly checked with control tubes of sterile broth which had been incubated an equal length of time.

TABLE 2

THE COMPARATIVE REACTIONS OF 25 CULTURES AND THEIR DESCENDANTS AFTER THE FIFTH AND TENTH PLATINGS

Culture Number	Dextrose			Lactose			Maltose			Dextrin			Saccharose		
	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+
4	+	—	—	+	—	—	+	—	—	+	—	—	+	—	—
5	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—
6	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—
7	+	—	+	+	+	+	+	—	+	+	+	+	+	+	+
8	+	—	+	+	—	+	+	—	+	+	—	+	+	—	—
9	+	+	+	—	+	—	+	+	+	+	+	—	+	—	—
10	+	+	+	+	—	+	+	+	+	+	+	+	+	—	—
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	—	—	+	+	+	+	—	+	—	+	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+
16	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	—	—	+	+	—	+	—	—	+	—	—	+	—	—	+
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	—	—	+	—	—	+	—	—	+	—	—	+	—	—	+
23	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—
25	—	+	—	—	+	—	—	+	—	—	+	—	—	—	—

Explanation: 0 = The original cultures; 5 = After the fifth plating; 10 = After the tenth plating.

[11] Jour. Bacteriol., 1917, 2, p. 403.

[12] Jour. Infect. Dis., 1912, 10, p. 253.

Table 2 shows the comparative fermentative reactions of the 25 cultures. Four of these cultures, 1, 8, 10 and 17, are virulent diphtheria bacilli which ferment not only dextrose, maltose and dextrin, but lactose and saccharose also, although the first three vary in this respect. Indeed, it is worthy of note that the fermentative reactions as investigated at 3 different stages of cultivation of these 25 cultures vary in many cases. Cultures 1, 2, 13, 17, 19, 20, 21 and 24 are the only ones whose titrations remained constant throughout the experiments. Table 3 gives the Wesbrook types present in the cultures at each titration together with their virulence and the types recovered at necropsy. Chart 1 shows further details of the fermentation tests while Chart 2 shows frequency curves derived from all the tests shown in Chart 1.

This study demonstrates that more than half the cultures investigated varied after successive platings in their power to produce acid from carbohydrates. Fermentation tests are of no value in testing virulence since even virulent cultures do not remain constant in their fermentative power. The frequency curves of Chart 2 indicate that virulent and nonvirulent diphtheria cultures as well as those members of the pseudodiphtheria group included in this study belong to one large group of organisms whose fermentative reactions vary around a single mean.

III. STUDY OF VIRULENCE

The control of the spread of diphtheria has become largely a matter of recognizing "carriers" of diphtheria bacilli by a demonstration of the presence of diphtheria organisms in the nose or throat. These bacilli are identified by their morphology, and conclusions in regard to their virulence are frequently based largely on the particular types of bacilli which are found. The relation of virulence to morphology is therefore of great importance.

There is general agreement that different strains of B. diphtheriae vary in their virulence for guinea-pigs, but there is considerable disagreement whether an individual culture can vary in virulence to any great degree. Rickards[13] reports finding that virulence tests made from different colonies of the same culture varied. He leaves the cause of this variation in virulence an open question, although he states that the cause was not a variation in the resistance of the guinea-pigs used.

[13] Am. Jour. Pub. Hyg., 1908, 18, p. 292.

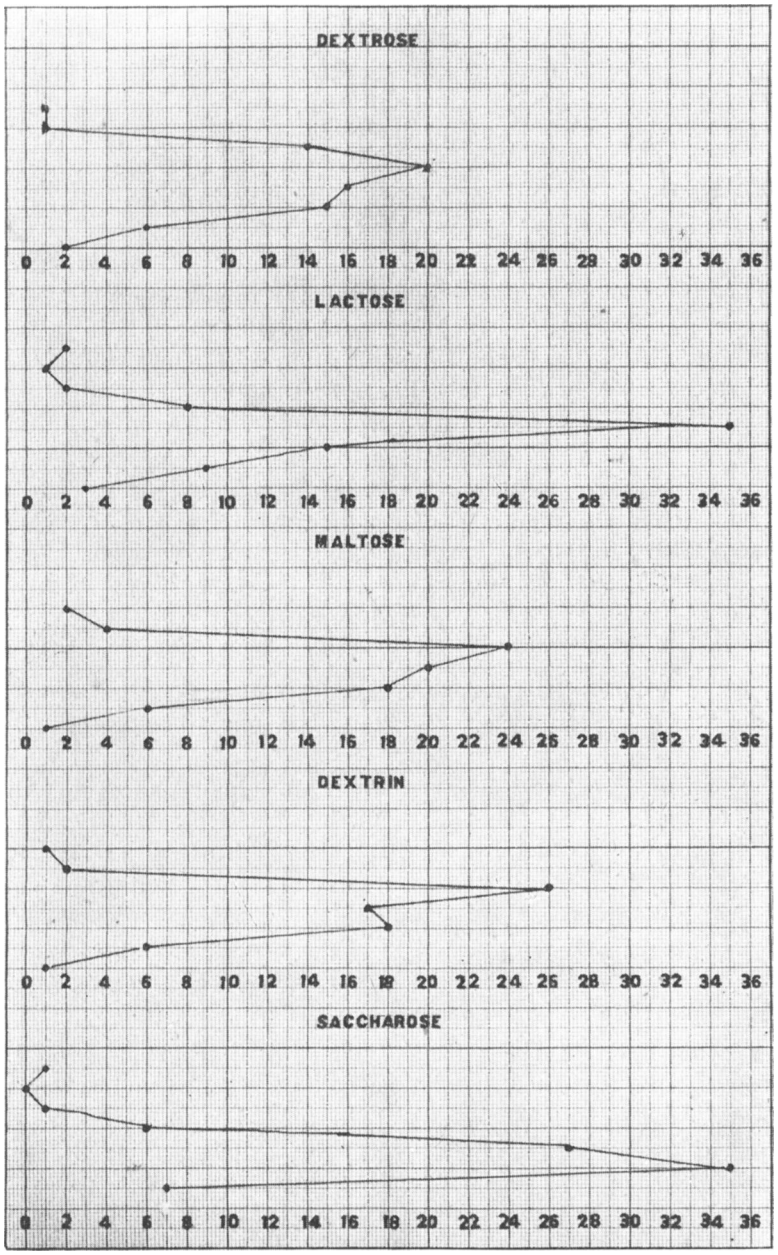

Chart 2.—Frequency curves of all 75 cultures shown in Chart 2.

Fermentative reactions of original cultures = ————.
Reactions of cultures selected after the fifth plating = —.—.—.—.
Reactions of cultures selected after the tenth plating = — — — — — —.

The epidemic of diphtheria in Newport, R. I., in the summer of 1917 well illustrates the fact that different strains of widely different degrees of virulence may occur in localities relatively close to each other. McCoy, Bolten and Bernstein[5] report that of 402 cases occurring in Newport there was only one death, while in Providence, R. I., there were during the same period 8 fatal cases among 62 reported, a case death rate of 12.90.

The difference of opinion in regard to variation in the virulence of the diphtheria bacillus and the importance of this question in the control of the disease led to the following study to attempt to determine whether the progeny of pure cultures of members of the diphtheria group vary in virulence from the parent culture and from each other.

The cultures used in this study were the same as those used in the studies on morphology and fermentative reactions. Virulence tests were made on the original cultures, the cultures selected after the fifth and after the tenth platings. Virulence tests were made by intraperitoneal inoculations of the culture grown in broth for 24 hours at 37 C. using guinea-pigs of weights varying from 200-350 gm. An amount of culture corresponding to 1% of the body weight of the animal was used for inoculation. The weight of the animals before inoculation and the daily weights for a period of 7 days following the inoculations were recorded. All pigs that died were examined at necropsy and material for cultures was taken from the principal organs and fluids. The types of organisms recovered at necropsy were compared with the types inoculated.

Table 3 shows the virulence of the 25 original cultures and their virulence after the fifth and tenth platings. Cultures 16, 17 and 24 were the only ones which proved virulent in each of the 3 tests. Five cultures proved virulent in 2 of the 3 tests. Of these, Cultures 5 and 6 are of peculiar interest because, although the original cultures were nonvirulent, the cultures selected after the fifth and tenth platings in each case proved virulent. Four cultures were virulent in 1 of the 3 tests, while the remaining cultures were consistently nonvirulent.

A comparison of the types inoculated with those recovered at necropsy shows that there is wide variation, a phenomenon which has been noted by other investigators. The types in Culture 5 after the fifth plating were B^2 C^2 D^2, yet when this culture was recovered at necropsy it showed ACC^1 DD^1 types. On the other hand, Culture 6, after the tenth plating contained CDD^2 types, but on recovery at necropsy C^2D^2 types occurred. Again, Culture 24, after the tenth plating contained only solid staining organisms when recovered at necropsy, although C^1 D^1 D^2 types were inoculated.

TABLE 3

THE TYPES, VIRULENCE AND TYPES RECOVERED AT NECROPSY OF 25 CULTURES OF MEMBERS OF THE DIPHTHERIA GROUP

	Original Culture			After Fifth Plating			After Tenth Plating		
	Type	Virulence	Type Recovered	Type	Virulence	Type Recovered	Type	Virulence	Type Recovered
1	ACC^1DD^1	+	D^2	A^1BCC^1	—		ACC^1D	+	CC^1D
2	$A^1B^1CC^1$	—		$ABCC^1$	—		CC^1DD^2	+	$C^1D^1D^2$
3	DD^1D^2	—		$A^1B^1C^1$	—		$CC^1D^1D^2$	—	
4	C^2	—		$A^2C^2D^2$	—		C^2	—	
5	DD^1D^2	—		$B^2C^2D^2$	+	$ACC^1DD^1D^2$	DD^1D^2	+	$C^1C^2D^2$
6	$C^1D^1D^2$	—		$CC^1DD^1D^2$	+	DD^1D^2	CDD^2	+	C^2D^2
7	$AC^1C^2D^2$	—		$CC^1C^2D^2$	—		A^2C^2	—	
8	$C^1D^1D^2$	+	$CC^1C^2DD^1D^2$	D^1D^2	—		DD^1D^2	—	
9	C^2D^2	—		D^2	—		D^1D^2	—	
10	$C^1D^1D^2$	+	CC^1DD^1	BB^1CC^1	—		CC^1DD^2	—	
11	$C^1D^1D^2$	—		C^1D^1	—		D^1D^2	+	$C^1D^1D^2$
12	D^2	—		D^2	—		D^2	—	
13	$B^1C^1C^2D^2$	—		D^2	—		E^2	—	
14	$A^1B^1C^1D^1$	+	$CC^1C^2DD^1D^2$	$C^1D^1D^2$	+	CDD^2	C^1D^1	—	
15	CC^1DD^1	—		$C^1D^1D^2$	+	ACC^2DD^2	D^1D^2	—	
16	D^1D^2	+	$C^1DD^1D^2$	D^1D^2	+	$C^1C^2D^2$	DD^1D^2	+	$CC^1C^2D^1D^2$
17	$AA^1C^1D^1$	+	C^2D^2	D^1D^2	+	C^1D^1	$C^1D^1D^2$	+	CDD^1D^2
18	$C^1C^2D^1D^2$	—		CC^2	—		$A^1C^1C^2D^2$	—	
19	D^1D^2	—		D^2	—		D^2	—	
20	D^2	—		D^2	—		D^2	—	
21	D^1D^2	—		D^1D^2	—		D^2	—	
22	D^1D^2	—		D^2	—		D^2	—	
23	D^2D^1	—		D^2	—		D^2	—	
24	$C^1DD^1D^2$	+	$A^1BB^1CC^1DD^1D^2$	CC^1DD^1	+	AA^1CC^1	$C^1D^1D^2$	+	C^2D^2
25	D^2	—		D^2	—		D^2	—	

A study of the relation of types inoculated to virulence brings out certain facts. Cultures containing only barred and solid staining types proved virulent, and in the case of the strain selected after the fifth plating of Culture 5 a culture containing only solid staining types proved virulent. While the most frequent morphologic picture of Culture 5 showed a mixture of granular, barred and solid staining types, the fact remains that at this particular stage of its cultivation solid staining forms only were present. It is also of interest that cultures containing granular forms were frequently nonvirulent. Those cultures which consisted of solid staining forms for the greater part of their cultivation were consistently nonvirulent.

In order to determine whether the results obtained in those virulence tests were due to variation in the virulence of the strains injected or to variation in the resistance of the guinea-pigs a further set of inoculations was made. Five pigs from different caviaries, of as nearly the same weight as possible, were inoculated with a broth culture of Number 1, selected after the fifth plating. This culture was grown in a large Erlenmeyer flask, and all the pigs were inoculated under as nearly the same conditions as possible. The original strain of Culture 1 and the strain selected after the tenth plating were virulent, while the strain selected after the fifth plating proved nonvirulent. This experiment should prove the truth of the first set of inoculations. Since none of the 5 pigs inoculated died it is evident that the culture varied in virulence rather than that the guinea-pigs varied in resistance.

This study shows that subcultures from a pure culture of members of the diphtheria group vary in virulence from the parent strain. The morphology of a virulent culture is often altered by passage through a guinea-pig. Granular and barred types are usually virulent, while solid staining types which retain this morphology through many generations are nonvirulent. Morphology is not, however, an index of virulence.

CONCLUSIONS

From this study of 25 cultures of members of the diphtheria group isolated from various sources the following conclusions appear justified:

From a biometric study of the fermentative reactions of members of the so-called diphtheria group it appears that they constitute a genetically related group of organisms.

Variations in morphology occur in subcultures derived from one parent strain.

Variations in fermentative reactions occur in subcultures derived from one parent strain.

Variations in virulence occur in subcultures derived from one parent strain.

The virulence of a strain is not closely correlated with its morphology.

The virulence of a strain is not correlated with its fermentative reactions.